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Article

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The identity of *Pentalonia nigronervosa* Coquerel and *P. caladii* van der Goot (Hemiptera: Aphididae) based on molecular and morphometric analysis

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Abstract

Pentalonia nigronervosa (*sensu* Hardy 1931) samples from banana and from Zingiberaceae and Araceae species exhibit fixed differences in DNA sequence in mitochondrial cytochrome oxidase subunit 1 ("DNA barcode") and in the nuclear gene elongation factor 1α, and have morphometric differences, including non-overlapping ranges in the length of the distal rostral segment. It is thus proposed that the name *P. nigronervosa* Coquerel be restricted to banana-feeding 'nigronervosa' specimens, and that the name *Pentalonia caladii* van der Goot be restored to full species status for specimens typically feeding on Zingiberaceae and Araceae.

Key words: DNA barcode, elongation factor 1α, species status

Introduction

The banana aphid, *Pentalonia nigronervosa* Coquerel (*sensu* Hardy 1931), is widely distributed throughout tropical and subtropical areas of the world, and is also found in greenhouses in North America and Europe (Blackman and Eastop 2000). In addition to banana and other species in the genus *Musa,* such as abaca (*M. textilis*, the source of Manilla hemp), it is found on various plant species in the order Zingiberales and in the family Araceae, including important food and ornamental plants such as cardamom (*Elettaria*), comb ginger (*Alpinia*), ginger (*Zingiber*), taro (*Colocasia*), *Caladium*, *Costus*, *Dieffenbachia*, *Hedychium*, *Heliconia* and *Xanthosoma* (Waterhouse 1987).

P. nigronervosa is economically important as the vector of banana bunchy top virus (BBVT) in Africa, East Asia, India, Australia and the Pacific Regions (Hu *et al*. 1996). BBVT is considered the most important disease of banana and related crops in the world (Dale 1987). The aphid is also capable of transmitting banana mosaic disease, papaya ringspot potyvirus and cardamon mosaic potyvirus (Hughes and Eastop 1991, Blackman and Eastop 2000). Reproduction is almost entirely asexual, the rare sexual forms being reported only from northeast India and Nepal (Bhanotar and Ghosh 1969, Blackman and Eastop 2000).

Coquerel (1859) first described *Pentalonia nigronervosa* on banana from the Indian Ocean island of Réunion. Subsequently, van der Goot (1917) described a second species, *Pentalonia caladii*, from *Caladium* in Java, without explicitly mentioning *P. nigronervosa* or providing characters distinguishing the two. Hardy (1931) believed the observed variation to be environmentally induced and placed *P. caladii* in synonymy. Although most authors since then have considered only one species, some authors recognized the variation within this species by separating forms "typica" and "caladii" (Eastop 1966; Eastop and Hille Ris Lambers 1976, Remaudière and Remaudière 1997). A few faunal lists have treated them as separate species (*e.g.* Cermeli 1990). Attempts have been made to study and explain morphological and biological variation among populations of the aphid (Rajan 1981; Padmalatha and Ranjit Singh 2001), but no firm conclusions were

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drawn. Two other species are currently placed in the genus *Pentalonia*: *P. gavarri* Eastop (1966), and *P. kalimpongensis* (A.N. Basu 1968).

In this paper, we apply molecular and morphometric approaches to the examination of variation in 'banana aphid' from various host plants in the Pacific Region, and present data supporting the restoration of *P. caladii* to full species status.

Material and methods

Thirty-eight collections (in 70% or 95% ethanol) of 'banana aphid' from banana and forty from Araceae, Zingiberaceae and Heliconiaceae were examined. Most were from Micronesia and Hawaii, but three collections from Florida and one from Australia were also included (Table 1). Specimens were cleared and mounted in Canada balsam on microscope slides after methods by Foottit and Maw (2000). Specimens with a WSU voucher identifier in Table 1 are deposited in Washington State University-Prosser Collections (WSU), the remainder in Canadian National Collection of Insects, Arachnids and Nematodes (CNC).

Twenty two measurements (listed in Table 2) were made on 162 apterous specimens taken from 32 collections (see last column of Table 1). An insufficient number of alate specimens was available among these collections for adequate analysis. Morphological measurements (all measurements in mm) were derived from image-measuring software by D. Allison (Pike *et al*. 2005). Distribution of individual characters was examined by plotting character values against body length. Principal component analysis (PCA; SAS Procedure PRINCOMP; SAS version 9.1.3; SAS Institute, Inc., Cary, North Carolina, USA) was used to assess overall patterns of variation. PCA iteratively calculates a linear combination of characters that maximizes the residual variation among all samples not explained by previous iterations, assuming all samples are from a single population (Tabachnick & Fidell 2006). Due to missing data for several characters (setal measurements, width of ultimate rostral article) or high correlation with other measurements (length of femur), the final measurement set was reduced to 15 characters (deleted characters indicated by asterisk in Table 2). Contribution of variables to the separation of classes revealed by these techniques was subsequently examined using canonical discriminant analysis (CDA; SAS procedure CANDISC). CDA calculates linear combinations of the variables that maximize the separation of the means of previously defined classes.

DNA sequence data for mitochondrial Cytochrome c oxidase subunit 1 (5' end) (COI; "DNA barcode") were obtained for samples for which suitably collected material was available (59 samples). DNA extraction, amplification and sequencing was done at the Biodiversity Institute of Ontario (Guelph, Ontario, Canada) using techniques described by deWaard *et al.* (2008). The primer pairs LepF and LepR, or the M13-tailed alternates LCO1490t1 and HCO2198t2 were used to amplify an approximately 700 bp DNA fragment of mitochondrial COI, which was subsequently sequenced in both directions using either LepF and LepR or M13F and M13R, yielding a 658 base pair "barcode". Primer sequences are available in BOLD — the Barcode of Life Data System (Ratnasingham and Hebert 2007), http://www.barcodinglife.org, "Published projects / View all primers" links. Sequence data and associated collection data are available on BOLD (public project "Banana-Ginger Aphid") and on GenBank (accession numbers GU140241 to GU140299). Pairwise distances were calculated using Kimura two parameter model (Kimura 1980) and the distance matrix visualized in a neighbour-joining tree (Saitou and Nei 1987) as implemented on BOLD.

Nuclear elongation factor-1α (EF1α) fragments were amplified using PCR with primers EF3 (5'-GAACGTGAACGTGGTATCAC-3') (Roderick, in Palumbi 1996) and EF6 (5'-TGACCAGGGTGGTTCAATAC-3') (von Dohlen *et al*. 2002). PCR products were sequenced directly using ABI BigDye v. 3.1 kit with an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA) giving a 916 base final sequence. Representative sequences for each unique haplotype of EF1α are available on GenBank under accession numbers GU130214 and GU130215.

TABLE 1. Material examined, with GenBank accession numbers for COI and EF1α sequences, and number of apterous individuals measured for morphometric analysis (note that length of ultimate rostral segment was measured in all sequenced individuals). Georeferenced collection data may be found in BOLD (Barcode of Life Database; http://www.barcodinglife.org). Vouchers of samples without a Washington State University accession number are held in Canadian National Collection with voucher number identical to the BOLD specimen identifier. (CNMI = Commonwealth of the Northern Mariana Islands; FSM = Federated States of Micronesia.)

host	Locality	WSU voucher	BOLD/CNC specimen ID	GenBank Accession No. CO1	GenBank Accession No. EF1α	Number of individuals measured
Zingiberaceae						
Alpinia purpurata	Guam: Chalan-Pago	A3R284	CNC#HEM052080	GU140250	n/a	**4**
Alpinia purpurata	Guam: Windward Hills	A5P003	CNC#HEM054584	GU140245	n/a	**11**
Alpinia sp.	Guam: Yigo	-	CNC#HEM057388	GU140271	n/a	–
Alpinia purpurata	FSM: Pohnpei: Kolonia	-	CNC#HEM057825	GU140266	n/a	–
Alpinia sp.	FSM: Pohnpei: Kolonia	A7K280	CNC#HEM057824	GU140262	n/a	**6**
Alpinia purpurata	USA: HI: Hawaii: Pepeekeo	A4H068	CNC#HEM051823	GU140247	*	–
Alpinia purpurata	USA: HI: Hawaii: Sheraton Resort	A7K315	CNC#HEM058113	GU140264	GU130214	**5**
Alpinia purpurata	USA: HI: Kauai: Kilauea	A5H003	n/a	n/a	n/a	**2**
Hedychium sp.	USA: HI: Hawaii: Kukio Beach	A7K323	CNC#HEM058119	GU140241	n/a	–
Hedychium coronarium	USA: HI: Kauai: Natl Bot.Garden	A4H014	CNC#HEM051881	GU140254	n/a	–
Hedychium coronarium	USA: HI: Hawaii: Rainbow Falls	A7K312	CNC#HEM058112	GU140260	*	–
Hedychium coronarium	USA: HI: Hawaii: Pepeekeo	A7K331	CNC#HEM058125	GU140246	*	**1**
Hedychium sp.	USA: Florida: Odessa	A7K341	CNC#HEM057500	GU140251	n/a	**5**
Zingiber sp.	Guam: Yona	A3R028	CNC#HEM050456	GU140265	*	**4**
Zingiber sp.	FSM: Pohnpei	A5R014	CNC#HEM054529	GU140248	*	–
Zingiber sp.	CNMI: Rota: airport	A3R053	CNC#HEM050481	n/a	n/a	–
Zingiber sp.	CNMI: Rota: Sabana	A3R048	CNC#HEM050476	n/a	n/a	**4**
Zingiber sp.	CNMI: Rota: Talakhya	A3R045	CNC#HEM050473	GU140269		**2**
Zingiber sp.	Palau: Koror: Ngerkebesang	A3R154	CNC#HEM050519	GU140261	*	**4**
Zingiber sp.	USA: HI: Kauai: Lawai	A4H027	CNC#HEM051894	GU140256	*	–
Zingiber sp.	USA: HI: Oahu: Waikiki	-	CNC#HEM059761	n/a	*	–
Zingiber sp.	USA: HI: Maui: Hana Road	A4H055	CNC#HEM051859	GU140259	*	–

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TABLE 1. (continued)

host	Locality	WSU voucher	BOLD/CNC specimen ID	GenBank Accession No. CO1	GenBank Accession No. EF1α	Number of individuals measured
Araceae						
Caladium sp.	Australia: New South Wales: Beecroft	-	CNC#HEM059845	GU140243	n/a	–
Colocasia esculenta	FSM: Pohnpei: COM	A7K296	CNC#HEM057834	GU140272	*	–
Colocasia esculenta	FSM: Pohnpei: Kolonia	A5R025	n/a	n/a	n/a	**3**
Colocasia esculenta	CNMI: Rota	A1R159	n/a	n/a	n/a	**10**
Colocasia esculenta	Palau: Peleliu: Peleliu	A3R304	CNC#HEM051902	GU140257	n/a	**4**
Colocasia esculenta	USA: HI: Hawaii: Kolakola State Park	A7K309	CNC#HEM058109	GU140268	*	–
Colocasia esculenta	USA: HI: Hawaii: North Kohala	-	CNC#HEM057377	GU140258	n/a	–
Colocasia esculenta	USA: HI: Hawaii: North Kohala	-	CNC#HEM057379	GU140270	n/a	–
Colocasia esculenta	USA: HI: Hawaii: North Kohala	-	CNC#HEM057381	GU140249	n/a	–
Colocasia esculenta	USA: HI: Maui: Hana Road	A4H058	CNC#HEM051862	GU140263	*	**4**
Cyrtosperma chamissionis	FSM: Pohnpei: Kolonia	A7K284	CNC#HEM057827	GU140244	n/a	**6**
Cyrtosperma chamissionis	FSM: Pohnpei: Kolonia	A7K285	CNC#HEM057828	GU140253	n/a	–
Cyrtosperma chamissionis	FSM: Pohnpei: Kolonia	A7K287	CNC#HEM057830	GU140255	n/a	–
Cyrtosperma chamissionis	FSM: Pohnpei: COM campus	A7K291	CNC#HEM057833	GU140252	n/a	–
Heliconiaceae						
Heliconia sp.	Guam: Yigo	-	CNC#HEM057389	GU140291	**	–
Heliconia sp.	FSM: Pohnpei: Kolonia	A7K288	CNC#HEM057831	GU140267	*	–
Heliconia pendula	CNMI: Tinian	A1R142	n/a	n/a	n/a	**6**
Heliconia pendula	Palau: Koror, Ngerkebesang	A1R203	n/a	n/a	n/a	**3**
Musaceae						
Musa sp.	Guam: Agat	A3R074	CNC#HEM050588	GU140294	n/a	**4**
Musa sp.	Guam: Dededo	98R158	n/a	n/a	n/a	**5**
Musa sp.	Guam: Inarajan	-	CNC#HEM057390	GU140292	**	–
Musa sp.	Guam: Inarajan	-	CNC#HEM057391	GU140285	n/a	–
Musa sp.	Guam: Mangilao	A3R131	CNC#HEM050645	GU140290	**	–
Musa sp.	Guam: Talofofo	A3R026	CNC#HEM050454	GU140286	GU130215	**4**
Musa sp.	Guam: Talofofo	A3R072	CNC#HEM050586	GU140293	n/a	**7**
Musa sp.	Guam: Talofofo	A3R083	CNC#HEM050597	GU140288	n/a	**4**
Musa sp.	Guam: Piti	A3R095	CNC#HEM050609	n/a	**	–
Musa sp.	Guam: Yigo	A3R096	CNC#HEM050610	n/a	**	–

continued next page

TABLE 1. (continued)

host	Locality	WSU voucher	BOLD/CNC specimen ID	GenBank Accession No. CO1	GenBank Accession No. EF1α	Number of individuals measured
Musa sp.	Guam: Yigo	-	CNC#HEM057382	GU140281	n/a	–
Musa sp.	Guam: Yigo	-	CNC#HEM057383	GU140283	n/a	–
Musa sp.	Guam: Yigo	-	CNC#HEM057384	GU140287	**	–
Musa sp.	Guam: Yigo	-	CNC#HEM057385	GU140296	n/a	–
Musa sp.	Guam: Yigo	-	CNC#HEM057386	GU140297	n/a	–
Musa sp.	Guam: Yigo	-	CNC#HEM057387	GU140295	n/a	–
Musa sp.	Guam: Yigo	A3R012	CNC#HEM050440	GU140284	**	–
Musa sp.	Guam: Yigo	A5P026	CNC#HEM054616	GU140280	n/a	**6**
Musa sp.	Marshall I: Majuro: Laura	-	CNC#HEM051977	GU140273	**	–
Musa sp.	FSM: Kosrae: Tolo	-	CNC#HEM051969	GU140279	**	–
Musa sp.	FSM: Kosrae: Tolo	-	CNC#HEM051972	GU140278	**	–
Musa sp.	FSM: Pohnpei: COM	A7K297	CNC#HEM057836	GU140298	n/a	–
Musa sp.	FSM: Pohnpei: COM	A7K298	n/a	n/a	n/a	**5**
Musa sp.	FSM: Pohnpei: COM	A7K299	CNC#HEM057837	GU140299	n/a	–
Musa sp.	FSM: Pohnpei: COM	A7K300	n/a	n/a	n/a	**5**
Musa sp.	FSM: Pohnpei: COM	A7K301	CNC#HEM057838	GU140274		–
Musa sp.	FSM: Yap: Gagil	A6P009	CNC#HEM055095	GU140282	n/a	**9**
Musa sp.	CNMI: Saipan	99N024	n/a	n/a	n/a	**5**
Musa sp.	CNMI: Tinian	-	CNC#HEM051909	GU140275	n/a	–
Musa sp.	CNMI: Tinian	A1R140	n/a	n/a	n/a	**5**
Musa sp.	CNMI: Tinian: San Jose	A3R062	CNC#HEM050482	GU140277	**	**4**
Musa sp.	Palau: Aimeliik: Aimeliik		CNC#HEM050516	n/a		
Musa sp.	Palau: Airai: Melengel	-	CNC#HEM050530	n/a	**	–
Musa sp.	Palau: Koror, Ngerkebesang	A1R198	n/a	n/a	n/a	**3**
Musa sp.	Palau: Koror: Ngerkebesang	-	CNC#HEM050520.2	GU140242	*	–
Musa sp.	USA: Florida: Hillsborough	-	CNC#HEM057951	GU140276	n/a	–
Musa sp.	USA: Florida: Gainesville	A7K340	CNC#HEM056689	GU140289	n/a	**7**
Musa sp.	USA: HI: Kauai: Lihue	93M002	n/a	n/a	n/a	**6**

* EF1α sequence identical to GenBank accession GU130214 (CNC#HEM058113, WSU A7K315).

** EF1α sequence identical to GenBank accession GU130215 (CNC#HEM050454, WSU A3R026).

Results

Morphometric data. Plots of most individual characters against body length reveal no particular deviations from continuous normal distributions. The largest specimens are from banana and the smallest from other hosts. The distribution of most other variables reflects this general size difference. However length of the ultimate rostral segment (URSL) is strongly bimodal, with a distinct gap between the two size classes (Figure 1). Furthermore, all specimens in the larger size class were taken from banana, while none of those in the smaller size were from this host. Summary univariate statistics, assuming two groups defined by host and rostral length, are given in Table 3.

TABLE 2. Variables measured. Variables marked with asterisk were omitted from final principal component analyses due to large number of missing values, difficulty of reliable measurement, or strong correlation with retained variables.

BL	Body Length, frons to cauda apex
HW	Head width, across eyes
A1_2	Combined length of antennal scape and pedicel (segments I and II)
A3, A4, A5	Length of first three flagellar segments (antennal segments III to V)
A6b	Length of base of distal antennal segment (VI), to distal margin of primary rhinarium
*PT	Length of processus terminalis of antennal segment VI
A3bd	Diameter of antennal segment III, at base
URSL	Length of distal (ultimate) rostral segment
*URSW	Width of distal rostral segment, at base
*F3	Length of hind femur
Tb3	Length of hind tibia
dTs3L	Length of distal segment of hind tarsus
*dTs3W	Maximal width of distal segment of hind tarsus
SiphL	Length of siphunculus
SiphW	Width of siphunculus at base
CdL	Length of cauda, from point in line with basal articulation
CdW	Width of cauda, at base
*HdSL	Length of longest anterior dorsal seta on head
*A3SL	Length of longest seta on antennal segment III
*Tb3SL	Length of longest seta on hind tibia

As would be expected, given this strong univariate effect, principal component analysis yields two discrete groups of points (not shown). In order to examine the patterns of variation among other characters, the PCA was repeated with URSL omitted. The difference in distribution across the common components of variation of the banana-collected specimens as compared to samples from other hosts remains evident (Figure 2). Contributions of the variables to the first three principal components, accounting for 81% of total variation, are given in Table 4. Component 1 reflects generalized body size (contribution by all variables positive and of approximately the same magnitude). The main contributions to component 2 are length of the distal segment of the hind tarsus and of the basal part of antennal segment 6, contrasted (opposite sign) with length of antennal segment 4 and length of the siphunculus.

Table 5 gives standardized total sample coefficients for canonical discriminant analysis with and without URSL. With URSL removed, the groups are distinguished by length of siphunculus and antennal segment 3 contrasting with width of head, length of tibia and length of antennal segment 4.

Revisting the univariate variable distributions, assuming two groups defined by URSL bimodality and by host, it is evident that, for a given body size, head width (Figure 3), and, to a lesser extent, tibial length, are

greater in the banana-feeding form, while antennal segment 4 and the siphunculus are relatively shorter when compared to general body size (Figure 4; use of the first principal component axis from Figure 2 as a measure of body size provides a clearer distinction than if body length alone is used as an indicator of size).

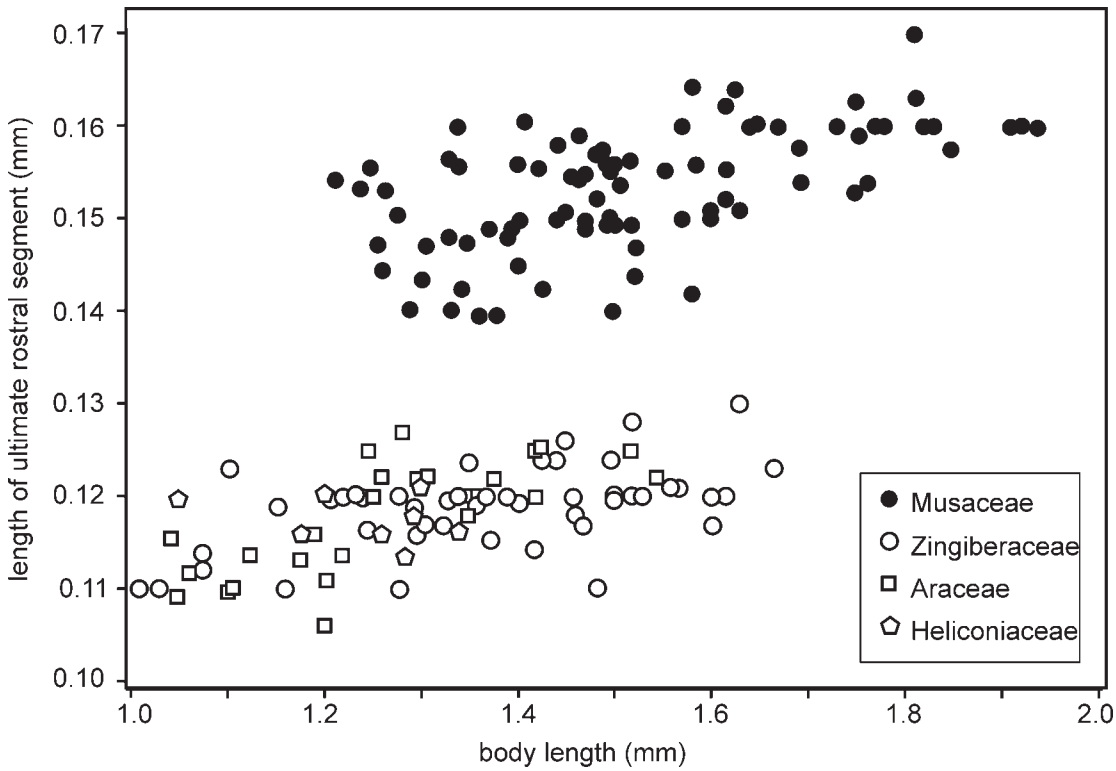

FIGURE 1. Plot of length of distal rostral segment versus body length of apterae. Symbols indicate host plant family of measured individual.

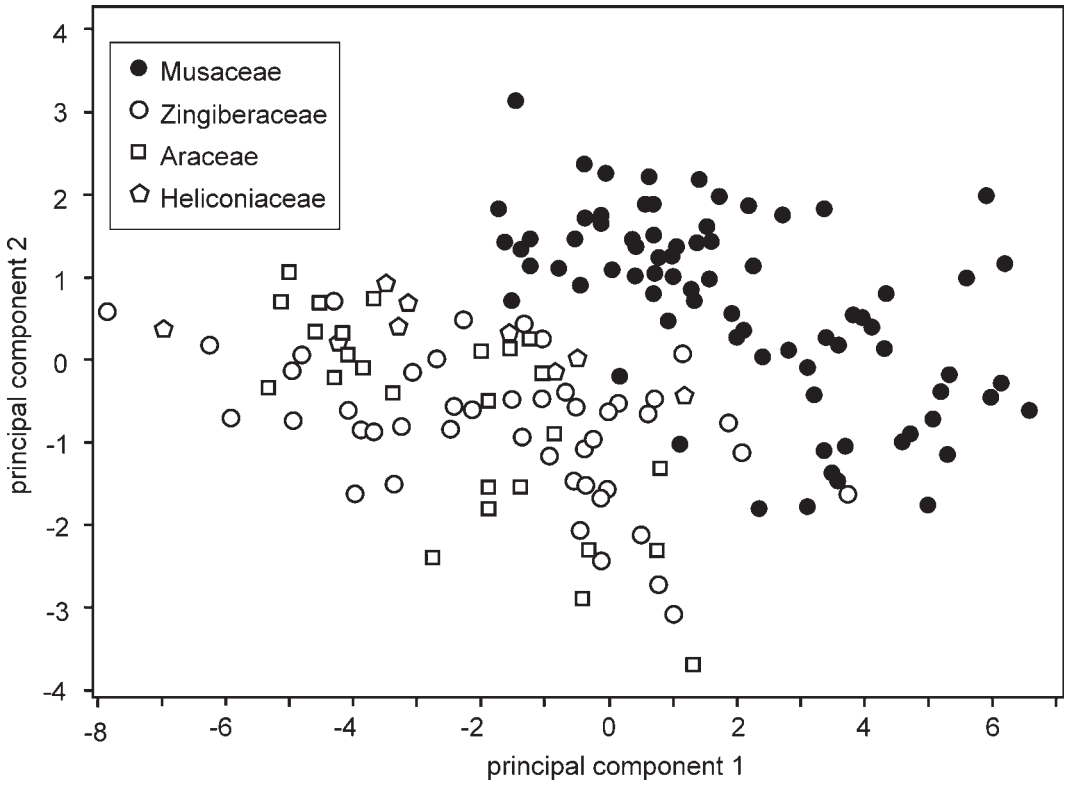

FIGURE 2. Plot of first two axes of principle component analysis, excluding distal rostral segment length from variable set. Symbols indicate host plant family of measured individual. First axis reflects generalized body size.

TABLE 3. Summary statistics for selected variables (see Table 2 for definition of variables) for the two groups of *Pentalonia nigronervosa sensu lato*. Groups are defined *a posteriori* based on the observed bimodal distribution of the last rostral segment illustrated in Figure 1. Within a group, the number of samples differs as a result of missing values due to mounting distortion or missing parts. Lengths in mm.

	Group 1 (on banana; *nigronervosa*)			Group 2 (on other hosts; *caladii*)		
variable	n	mean (range)	standard deviation	n	mean (range)	standard deviation
BL	89	1.52 (1.21 – 1.94)	0.175	89	1.33 (1.01 – 1.67)	0.161
URSL	92	0.153 (0.137 – 0.170)	0.007	84	0.118 (0.106 – 0.130)	0.005
HW	91	0.447 (0.393 – 0.500)	0.027	89	0.392 (0.330 – 0.461)	0.025
A3	91	0.344 (0.268 – 0.500)	0.043	89	0.297 (0.200 – 0.395)	0.036
A4	91	0.191 (0.120 – 0.320)	0.035	89	0.185 (0.118 – 0.270)	0.033
A5	90	0.18 (0.13 – 0.22)	0.018	89	0.16 (0.11 – 0.22)	0.022
A6b	88	0.092 (0.070 – 0.104)	0.007	88	0.081 (0.066 – 0.081)	0.007
PT	78	0.593 (0.416 – 0.700)	0.065	85	0.493 (0.270 – 0.620)	0.075
Tb3	90	1.008 (0.744 – 1.186)	0.090	89	0.872 (0.600 – 1.060)	0.091
dTs3L	89	0.074 (0.058 – 0.094)	0.007	87	0.062 (0.042 – 0.092)	0.009
SiphL	92	0.308 (0.252 – 0.370)	0.030	89	0.296 (0.220 – 0.370)	0.029
SiphW	90	0.092 (0.063 – 0.123)	0.012	88	0.070 (0.050 – 0.106)	0.010
CdL	91	0.107 (0.069 – 0.130)	0.012	89	0.090 (0.068 – 0.110)	0.009
CdW	89	0.071 (0.050 – 0.085)	0.007	87	0.057 (0.042 – 0.075)	0.006

TABLE 4. Coefficients for first three principal components (together accounting for 81% of total variation), with length of ultimate rostral segment omitted from the analysis. Variable names are defined in Table 2. Proportion of total variation accounted for by each component is given in the last row.

variable	Component 1	Component 2	Component 3
BL	0.283	-0.091	-0.254
HW	0.297	0.139	-0.225
a1_2	0.282	0.116	0.373
A3	0.304	-0.176	0.053
A4	0.230	-0.470	0.269
A3bd	0.232	-0.108	-0.525
A5	0.290	-0.220	0.029
A6b	0.204	0.305	-0.370
Tb3	0.313	-0.040	0.031
dTs3L	0.180	0.515	0.399
SiphL	0.264	-0.357	0.223
SiphW	0.274	0.248	-0.060
CdL	0.291	0.026	-0.089
CdW	0.254	0.308	0.182
proportion of total variation	65%	11%	5%

Molecular data. COI sequences fall into two discrete haplotype groups (Figure 5). All sequences in 'group 1' are identical, while pairwise distances within the second group range from 0 to 0.366% (mean 0.020%, standard deviation 0.07). The two groups differ by over 3% sequence divergence (pairwise between-

group distances range from 3.34 to 3.77%, mean 3.31%, standard deviation 0.08), resulting in one amino acid change (methionine – leucine alternation). One haplotype ('group 1') corresponds mainly to banana-feeding samples (of 27 samples with this haplotype, 26 were collected from banana, 1 from *Heliconia*), while the other group ('group 2') corresponds mainly to samples taken from other hosts (1 from banana, 31 from other hosts).

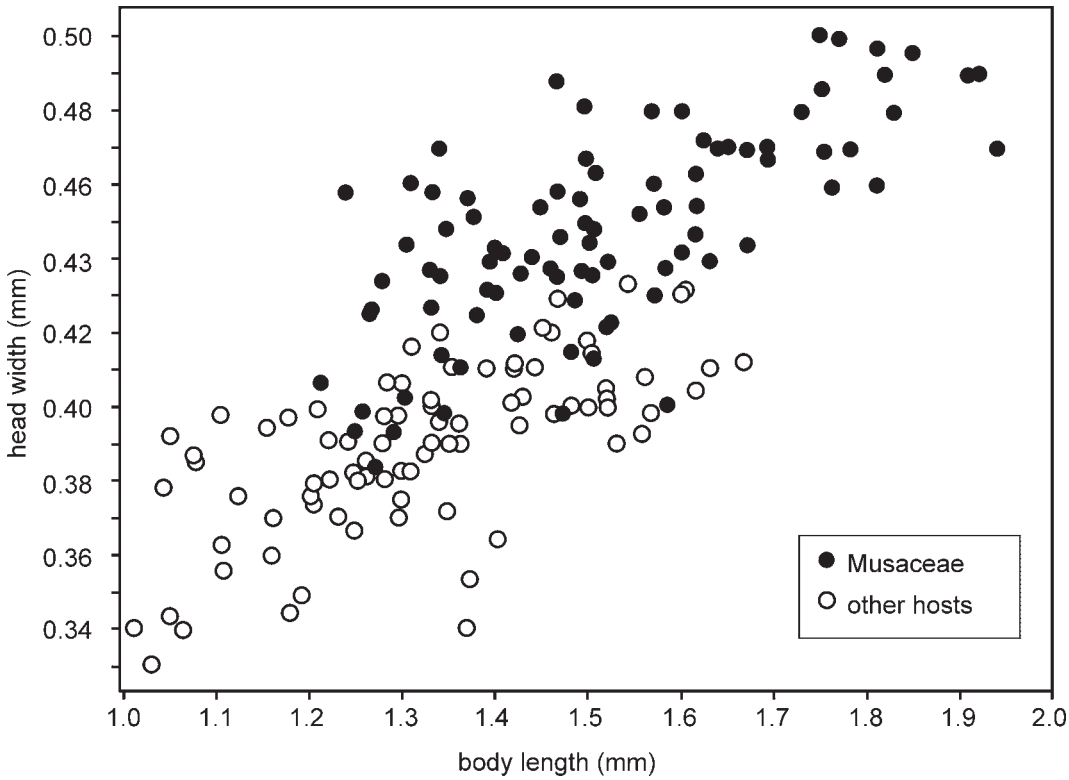

FIGURE 3. Head width plotted against body length with data points labelled by host plant (lengths in mm).

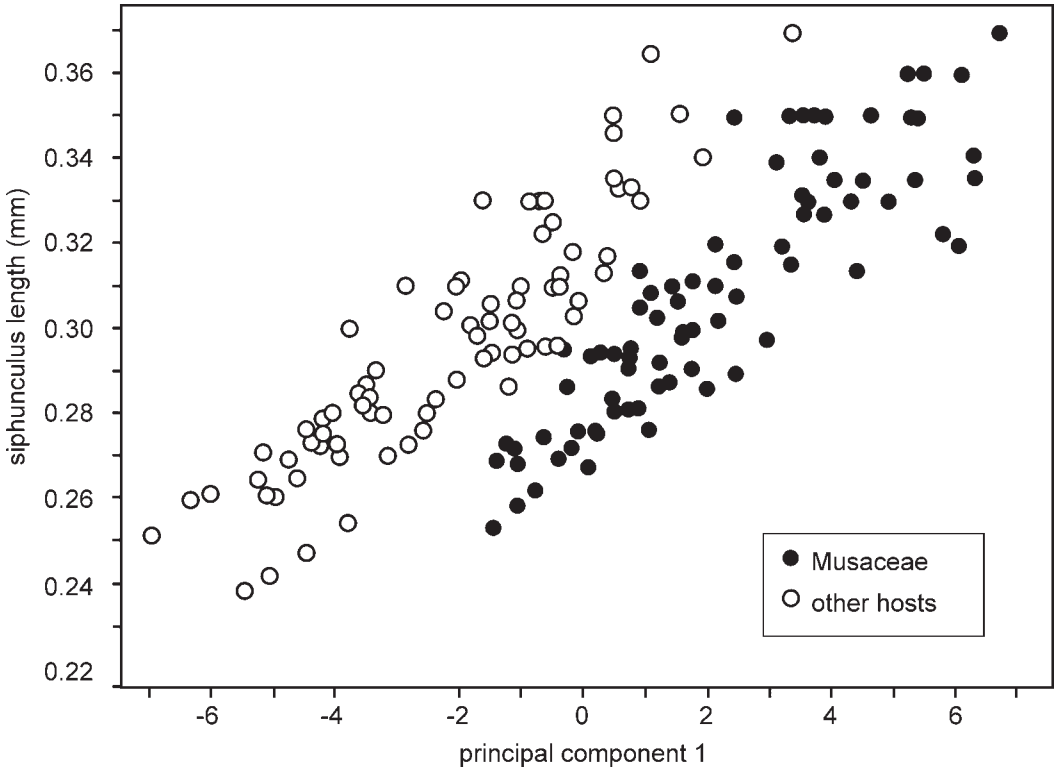

FIGURE 4. Siphunculus length (in mm) plotted against first principal component (without length of ultimate rostral segment) as estimate of generalized body size. Data points labelled by host plant.

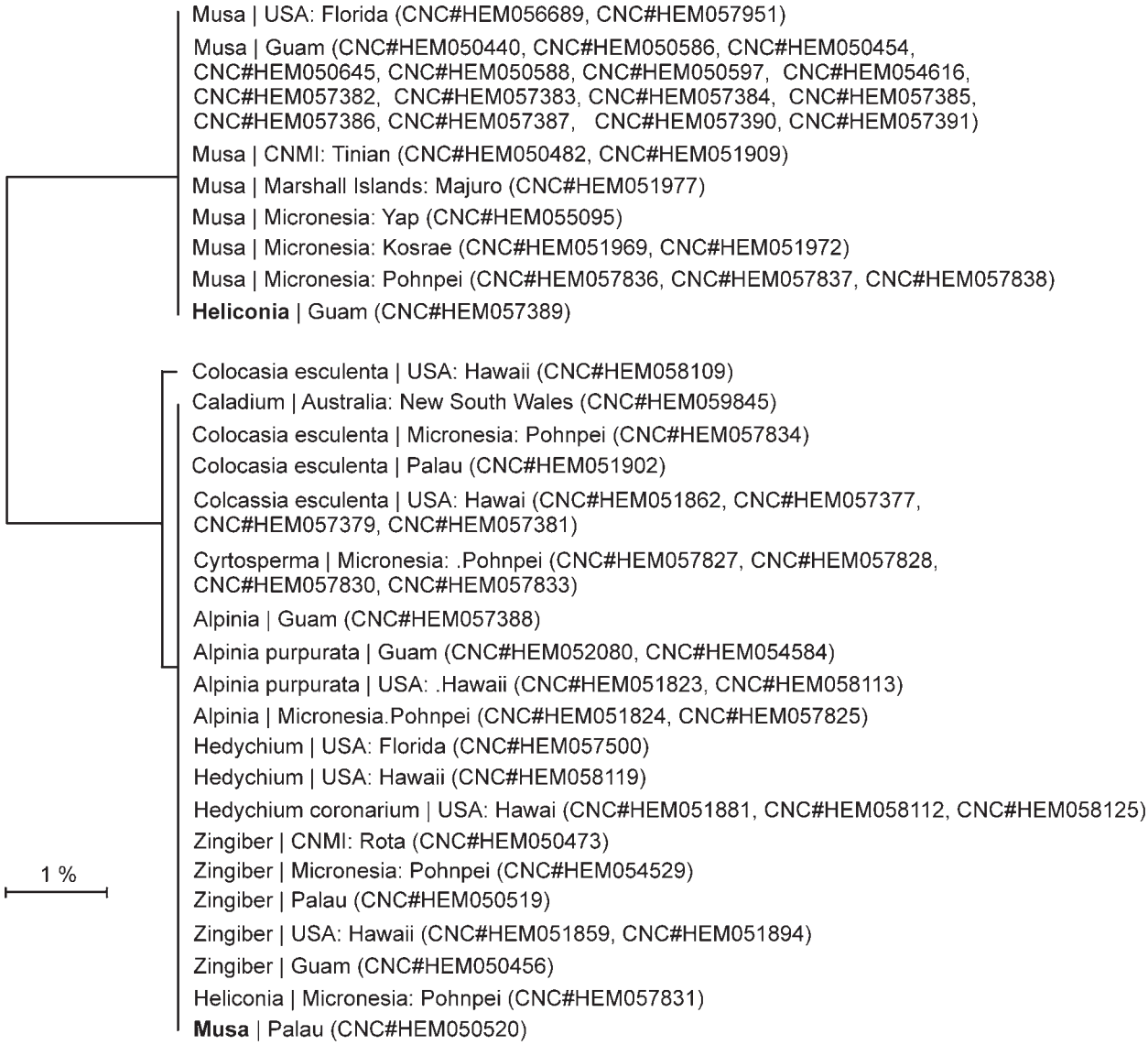

FIGURE 5. Neighbor-joining tree (Kimura 2 parameter model) for CO1 (barcode) sequence data, indicating host plant, geographic area and BOLD specimen identifier for each sample. Host names in bold faced text indicate exceptions to the overall host-associated grouping of sequences. (CNMI = Commonwealth of Northern Mariana Islands.)

EF1α sequences also fall into two groups based on three fixed differences (one C/T transition in the second exon, a 4 base insertion/deletion in the second intron, and a one base insertion/deletion in the third intron), with the partition corresponding exactly to that obtained from COI data. No heterozygosity is present in the examined material.

In all samples with both molecular and morphometric data available, 'group 1' corresponds to the morphologically defined 'banana group' with long rostrum, and 'group 2' to the short-rostrum group, without exception. The length of the ultimate rostral segment was measured on voucher specimens for all sequence data. In all of these specimens, group 1 corresponds to the long-rostrum form, and group 2 to the short-rostrum form (including the one banana sample on the 'wrong' host)

Discussion

The concordance of molecular data with the morphological data and normal host preference, over a wide geographic range, demonstrates that the morphological differences between the two groups are genetically based, rather than host-induced phenotypic responses.

The most recent catalogues (Remaudière & Remaudière 1997; Eastop and Hille Ris Lambers 1976) list *Pentalonia caladii* van der Goot as a 'form', but this is not a formally recognized taxonomic category and most authors have ignored the distinction or recognized it only in passing. Although reproduction is almost entirely asexual, presenting problems for application of species concepts, from an operational point of view, recognition of the two entities is most unambiguously achieved by giving them species status.

TABLE 5. Total sample standardized coefficients of variables for canonical discriminant analysis with (column A) and without (column B) length of ultimate rostral segment (URSL).

	A	B
BL	-0.282	-0.061
HW	0.358	0.969
a1_2	-0.208	0.142
A3	0.246	0.715
A4	-0.177	-0.706
A5	-0.117	0.416
A6b	-0.091	0.172
A3bd	-0.270	-0.359
URSL	3.642	–
Tb3	0.304	0.836
dTs3L	0.235	0.030
SiphL	-0.746	-1.513
SiphW	0.183	0.393
CdL	-0.045	0.168
CdW	0.699	0.554

The original description of *P. nigronervosa* is based on samples from banana. No type material apparently exists (Hille Ris Lambers, 1949), measurements were not given, and the description could apply equally to both forms. No other material from Réunion, the type locality, has been examined. However, given that most economic literature under the name *P. nigronervosa* applies to banana culture, it is in the interest of stability to make the reasonable assumption that this name should apply to the typical banana-feeding form. The description of *P. caladii* does not explicitly distinguish the species from *P. nigronervosa*, no rostral measurements are given, and the existence and location of type material is unknown. However, the short-rostrum form is likely the same as van der Goot's species. This is in accord with the interpretation of Noordam (2004), who distinguished typical *P. nigronervosa* from form "caladii" in Java (which is the origin of van der Goot's material) using non-overlapping differences in rostral length and host associations that correspond well with our results. The sexual female described by Bhanotar and Gosh (1969) from West Bengal, collected on *Curcuma domestica* (Zingiberaceae), has an ultimate rostral segment length of 0.116 mm. Thus it appears that this specimen is best considered a member of the ginger-feeding group. Although almost all of our samples are from Pacific Islands, the confirmation given by specimens from Florida and Australia (also specimens from a Zingiberaceae species in a greenhouse in Canada, data not shown), and the consistency with the Noordam's (2004) interpretation of the species in Java and with the ovipara from India lead us to believe that our results are generally applicable world-wide.

The magnitude of the difference in COI barcode sequence between the two species is rather large for such similar taxa, but not inconsistent with other congeneric aphid species pairs (compare data for other aphids in Foottit *et al.* 2008). The COI sequence is quite unlike that of all other Macrosiphini sequenced to date, including members of such potentially related genera as *Idiopterus*, *Neotoxoptera*, *Micromyzus* and *Myzus* (R.G. Foottit, unpublished data). It is possible that the barcode sequence obtained is in fact that of a nuclear

pseudogene or genetic material transferred to an endosymbiont, rather than true mitochondrial COI, but the absence of reading frame shifts, stops, and evidence of background true COI sequence suggests that this is not the case. It is also possible that one or both forms are of hybrid origin, with different maternal parentage, and thus each carrying the mitochondrial genome of different parental species. Some other mechanism for interspecies transfer of mitochronrial DNA or selective disequilibrium (such as *Wolbachia* infection; see Hurst and Jiggins 2005) may also have played a role. Whatever the origin of the large difference, the two COI haplotypes are maintained separately by the continuous asexual reproduction prevalent in both species. In contrast, the difference in EF1α sequence is modest and restricted to the non-coding introns. Amplification and dispersal of particular source populations by asexual reproduction also serves to explain the within-group uniformity exhibited by the EF1α sequences, although more extensive future sampling of the nuclear genome may demonstrate more diversity than observed here. It would be enlightening to obtain samples from populations with demonstrated sexual reproduction, and from the two other known *Pentalonia* species, for comparison.

The difference in host preference suggests that there may be other biological differences. Thus it is essential that biological and ecological studies on this economically important aphid specify the species of *Pentalonia* being studied. Past literature dealing with banana aphid and banana bunchy top virus management fail to adequately recognize these distinctions (*e.g.* Raymundo and Bajet 2000, Robson *et al.* 2007), although inferences may be made from the source host of the samples studied. While *P. nigronervosa* is a well known vector of BBTV, it is not known if *P. caladii* also acts as a vector, or if the virus can be transferred to the plants that it infests. Furthermore, given that one collection of each species was found on *Heliconia*, that one sample of *P. caladii* was taken on banana (at the same locality and time as a collection on ginger), and that material from other *Musa* species (especially abaca) was not examined, the full normal host range of the two species, and the frequency of occurrence on uncharacteristic hosts is not yet clear. Although the joint distribution is known, the exact geographic distribution of the two species also remains to be determined. All of these factors have an impact on effective management of the *Pentalonia* species and BBTV.

Conclusion

The fixed differences between the two forms treated here are sufficient to warrant the re-establishment of *Pentalonia caladii* van der Goot as a full species. *Pentalonia nigronervosa* Coquerel is thus restricted to the form typically feeding on banana, but occasionally found on other hosts (one instance on *Heliconia* in this study). *P. caladii* typically feeds on Zingiberaceae and Araceae, occasionally on *Heliconia* and *Musa* species (one instance on each found in this study).

P. caladii is easily separated from *P. nigronervosa* by the shorter distal rostral segment (less than 0.13 mm). Other measurements for apterae are given in Table 3. The key given by Noordam (2004) adequately separates the two species. *P. nigronervosa* and *P. caladii* are both distinguished from *Pentalonia kalimpongensis* (A.N. Basu 1968), which also feeds on Zingiberaceae, by the longer terminal process of the antenna (ratio of length of process to basal part of distal segment usually greater than 5.0 in *P. nigronervosa* and *P. caladii*, less than 4.5 in *P. kalimpongensis*), and by the fusion of the radius with the medial vein in alate specimens (approaching but not contacting the medius in *P. kalimpongensis*). Specimens of the grass feeding species, *Pentalonia gavarri* Eastop (1967), have longer siphunculi (more than 3 times length of ultimate rostral segment, versus less than 3 times in *P. nigronervosa* and *P. caladii*) and more setae on abdominal tergum 8 (4 or 5 rather than 2 setae).

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